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Purification and characterization of agaropectin sulfatase produced from *Sphingomonas* AS6330.

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INTRODUCTION

Marine microorganisms have a diverse range of enzymatic activity and are capable of catalyzing various biochemical reactions with diverse enzymes. There have been several studies reporting that various sulfatases isolated from such bacteria as *Klebsiella pneumoniae* (Miech *et al.*, 1998), *Salmonella typhimurium* (Henderson and Milazzo, 1979; Murooka and Harada, 1981), *Serratia marcescens* (Murooka *et al.*, 1980), *Pseudomonas aeruginosa* (Delisle and Milazzo, 1970), and *Comamonas terigena* (Fitzgerald and Cline, 1977). Agaropectin sulfatese (E.C. 3.1.6.1) hydrolyzes arylsulfate esters to aryl compounds and inorganic sulfate by substitution/elimination mechanism, leading to a covalently bound sulfated enzyme intermediate and aldehyde (Waldow *et al.*, 1999). We screened marine bacteria producing an agaropectin sulfatese, and found *Sphingomonas* AS6330, a new strain of *Sphingomonas* sp. nov. Objective of this research is to establish a purification protocol for the agaropectin sulfatese from *Sphingomonas* AS6330 and to characterize its enzymatic properties.

MATERIALS AND METHODS

Sphingomonas AS6330 was grown in a rich medium in 8 L laboratory bioreactor under constant cultivation conditions of 30℃ and pH 7.0. The agaropectin sulfatese activity was determined as p-nitrophenyl release from p-nitrophenyl sulfate (NPS). agaropectin sulfatese from *Sphingomonas* AS6330 was purified through ionic exchange chromatography, gel filtration, and isoelectricfocusing. The molecular weight of the isolated agaropectin sulfatese was determined using gel filtration. The optimal pH for the determination of agaropectin sulfatese activity were

screened with a series of buffers whose pHs ranges from 4.0 to 10.1. The optimal temperatures of agaropectin sulfatese activity were measured at pH 7.0 over a temperature range of 25 - 70℃. All inhibitor solutions of phenylmethylsulfonyl fluoride (PMSF), leupeptin, pepstatin, iodoacetate, and ethylene diamine teteraacetate (EDTA) were prepared in order to see their effects on the agaropectin sulfatese activity.

RESULTS

The agaropectin was purified 184,000-fold with 16.58% yield over the culture medium. Specific acitity of the purified sulfatese showed 127.6 U per mg protein at 45℃ and pH 7.0. The molecular weight of the enzyme was estimated to be 40 kDa. The enzyme showed an optimum reaction condition at pH 7.0 and 45℃. PMSF (1 mM), pepstatin (1 mM), and EDTA significantly decreased the activity. However, the enzyme sustained 80% and 85% of its original activity by adding of 1 mM leupeptin and iodoacetate, respectively. With the reaction of agar with agaropectin sulfatese in the ratio of $1:10^{-6}$ (w/w) for 2 hr at 50℃, gel strength of agar was increased to 2.3 fold and 97% ($p<0.01$) of sulfate in agar was removed. The result suggests that the agaropectin sulfatese could be applicable to the production of value-added products such as electrophoresis grade agarose.

REFERENCES

Miech C., T. Dierks, T. Selmer, K. von Figura, and B. Schmidt. 1998. Arylsulfatese from *Klebsiella pneumoniae* carries a formylglycine generated from a serine. *J. Biol. Chem.* **273**:4835-4837.

Henderson M.J., and F.H. Milazzo, 1979. Arylsulfatese in *Salmonella typhymurium*: detection and influence of carbon source and tyramine on its synthesis. *J. Bacteriol.* **139**:80-87.

Murooka Y., M.H. Yim, and T. Harada. 1980. Formation and purification of *Serratia marcescens* arylsulfatese. *Appl. Environ. Microbiol.* **39**:812-817.

Murooka Y., M.H. Yim, and T. Harada. 1980. Formation and purification of *Serratia marcescens* arylsulfatese. *Appl. Environ. Microbiol.* **39**:812-817.

Delisle G., and F.H. Milazzo. 1970. The isolation of arylsulphatase isoenzymes from *Pseudomonas aeruginosa*. *Biochim. Biophys. Acta.* **212**:505-508.

Fitzgerald J.W., and M.E. Cline. 1977. The occurence of an inducible arylsulphatase in *Comamonas terrigena*. *FEMS Microbiol. Lett.***2**:221-224.

Waldow A, B. Schmidt, T. Dierks, R. von Bulow, and K. von Figura. 1999. Amino acid residues forming the active site of arylsulfatese A. *J. Biol. Chem.* **274**:12284-12288.